4. Frederickson, C.J., et al (2006) *Experimental Neurology* **198,** 285–293
5. Fiskum G., et al. (2008) Mitochondria and Oxidative Stress in Neurodegenerative Disorders: *Annals of the New York Academy of Science.* **1147,** 129–138
6. Weiss, J.H., et al. (2000) *Trends in Pharmacological Science* **21,** 395–4017.
Jiang, D., et al. (2001) *Journal of Biological Chemistry* **276,** 47524–47529
8. Gazaryan, I.G., et al. (2007) *Journal of Biological Chemistry* **282,** 24373–24380
9. Bozym, R.A., et al. (2006) *ACS Chemical Biology* **1,** 103–111.

236-Pos Board B36

Isoflurane Enhances Reactive Oxygen Species Generation via Attenuation of Complex I

Naoyuki Hirata, David Stowe, Zeljko Bosnjak, Martin Bienengraeber.

BACKROUND: Reactive oxygen species (ROS) mediate anesthetic-induced protection of the heart from ischemia and reperfusion injury (anesthetic preconditioning, APC), but the precise role and mechanism of ROS generation remain unknown. In this study, we tested if a mitochondria-targeted mimetic of superoxide dismutase (mito-tempol, MT) can abolish the reduction in myocardial infarct size afforded by the volatile anesthetic isoflurane. Further, we investigated the mechanism by which isoflurane generates ROS in isolated mitochondria and submitochondrial particles. METHODS: Rats received 0.9% saline (control) or 3.0 mg/kg MT with or without exposure to 1 minimum alveolar concentration isoflurane for 30 min. Myocardial infarction was performed by left anterior descending artery occlusion for 30 min followed by 2 h reperfusion. Infarct size was measured by patent blue and triphenyltetrazolium chloride staining. Mitochondrial ROS production was measured spectrofluorometrically in isolated mitochondria and submitochondrial particles using the fluorescent probe amplex red. The effect of isoflurane on mitochondrial respiratory complex enzyme activities was determined spectrophotometrically in cholic acid-solubilized mitochondria. RESULTS: APC reduced infarct size of the left ventricular area at risk (mean ± SD=40 ± 9%) relative to the control (60 ± 4%). MT abolished cardioprotection (60 ± 9%) afforded by APC. Isoflurane enhanced mitochondrial ROS production induced by antimycin A or oxidized ubiquinone in the presence of substrates pyruvate and malate, but not succinate. Isoflurane also produced ROS at Complex I in the absence of any inhibitors in submitochondrial particles. Mitochondrial respiration and electron transport chain complex assays revealed that isoflurane only inhibits complex I activity. CONCLUSIONS: These results highlight that ROS are critical for APC. Moreover, the results indicate that isoflurane produces ROS at complex I and enhances ROS generation at complex III of the respiratory chain via its effect to attenuate complex I activity.

237-Pos Board B37

Relating Mitochondrial Flickering to Whole-Cell Oscillations in Cardiac Mitochondrial Networks

Melissa M. Nivala, Paavo Korge, James N. Weiss, Zhilin Qu.

The membrane potential of individual mitochondria has been shown to "flicker" randomly, in conjunction with the release of reactive oxidative species (ROS), such as superoxide flashes demonstrated recently in experiments. Under conditions of laser-induced oxidative stress, mitochondrial depolarization waves and oscillations, attributed to ROS-induced ROS release (RIRR) have been observed in cardiac myocytes and simulated in computer models. However, how asynchronous single mitochondrial flickering transitions to organized mitochondrial waves and oscillations is unknown. In this study, we developed a simplified, agent-based model of mitochondrial networks. In a single mitochondrion, superoxide production was modeled as a bistable process, where low oxygen triggers a higher percentage of oxygen shunted to superoxide. Mitochondrial channels (IMAC and mPTP) open stochastically in response to superoxide inside the matrix and cytoplasm (RIRR), causing flickering in the membrane potential and release of superoxide into the cytoplasm. In a globally coupled network simulating a well-mixed population of isolated mitochondria in a cuvette, we recapitulated experimental findings from isolated heart mitochondria showing that membrane potential oscillated when oxygen reached a critically low level. The oscillations were a self-organizing behavior arising from synchronization of flickering mitochondria at low oxygen levels. Using the same mitochondrial model in a locally coupled network to simulate the mitochondrial network of a myocyte, we found that the frequency of randomly flickering increased as ROS levels increased. At a critical level, a phase transition occurred in which self-organizing clusters of depolarized mitochondria propagated through the network, resulting in ROS waves and whole-cell oscillations. Our model predicts that this phase transition can be induced through either excessive laser light or hypoxic conditions.

238-Pos Board B38

The Effects of Idebenone on Mitochondrial and Cellular Bioenergetics

Valentina Giorgio, Valeria Petronilli, Maurizio Prato, Anna Ghelli, Michela Rugolo, **Paolo Bernardi**.

Idebenone [2,3-dimethoxy-5-methyl-6(10-hydroxydecyl)-1,4-benzoquinone] is a synthetic short-chain analogue of coenzyme Q10 (CoQ10). A variety of quinones (including idebenone) have also been shown to affect the mitochondrial permeability transition pore (PTP), a high-conductance inner membrane channel modulated by the proton electrochemical gradient and by many signaling molecules. The PTP links oxidative stress to cell death, and may be involved in the pathogenesis of Leber's hereditary optic neuropathy (LHON) and possibly to other conditions with complex I deficiency. Given these complex effects of idebenone, we have investigated its effects on bioenergetics and PTP modulation in intact cells. Our results indicate that: (i) idebenone promotes CsA-sensitive opening of the PTP and subsequent loss of pyridine nucleotides; (ii) dithiothreitol prevents PTP opening and its detrimental consequences; (iii) idebenol does not cause PTP opening, and stimulates electron transfer at complex III of the respiratory chain; and (iv) idebenol-stimulated respiration is coupled to ATP synthesis both in rotenone-treated normal cells and in RJ206 cells (harboring the 3460/ND1 LHON mutation) and XTC.UC1 thyroid oncocytoma cells (bearing a disruptive frameshift mutation in the MT-ND1 gene, which impairs complex I assembly). Thus, under proper experimental conditions idebenol, can be a useful tool to bypass complex I defiencies.

239-Pos Board B39

Similar Inhibition of the Mitochondrial Permeability Transition Pore Opening by Intralipid and Cyclosporine-A after Ischemia Reperfusion

Jingyuan Li, Jean C. Bopassa, Kaveh Navab, Andrea Iorga, Rangarajan D. Nadadur, Parisa Partownavid, Siamak Rahman, Mansoureh Eghbali.

It has been shown that intralipid(ILP) protects the heart against ischemia reperfusion (I/R) injury. However the mechanism of its action is not clear. Here we investigated whether ILP-induced cardioprotection is mediated by inhibition of the mitochondria permeability transition pore (mPTP) opening. We compared the effect of ILP with cyclosporine A (CsA), a well known inhibitor of mPTP. Isolated mouse hearts were subjected to 20 minutes of global ischemia followed by 10 minutes reperfusion with i)Krebs Henseleit buffer (CTRL),ii) additional 1% ILP or iii)1.5μM CsA.The hearts which were not subjected to ischemia/reperfusion served as sham. The calcium retention capacity (CRC) was measured in isolated cardiac mitochondria in the absence or after addition of 2 μM of CsA in the cuvette. DHE staining of the heart tissue sections was used to measure the production of reactive oxygen species (ROS). The CRC was significantly lower in CTRL compared to sham (1.5 ± 0.2 vs 3.7 ± 0.2μM/mg protein, $p<0.05$). However, the treatment with ILP or CsA significantly improved the CRC compared to CTRL(2.8 ± 0.1 in ILP, 2.6 ± 0.3 μM/mg protein in CsA). Addition of CsA directly in the cuvette, resulted in a similar significant increase in CRC between ILP and CsA groups (4.5 ± 0.3 *vs.* 4.2 ± 0.5μM/mg protein, $p>0.05$). However, the increase in CRC in CTRL and sham groups after addition of CsA in vitro were much higher (2.2 and 1.6 fold increase separately). ROS production was significantly lower in ILP and CsA group compared to CTRL (normalized to CTRL, 1.00 ± 0.03 in CTRL *vs.* 0.57 ± 0.04 in ILP, $p<0.05$). In conclusion, intralipid inhibits the opening of the mPTP in a similar fashion as CsA via a CypD-dependent mechanisms. This inhibition resulted in decreased sensitivity of mPTP to calcium overload and reduction of ROS production.

240-Pos Board B40

The Embryonic Mitochondrial Permeability Transition Pore Controls Cardiac Myocyte Mitochondrial Maturation and Differentiation

Jennifer R. Hom, Rodrigo A. Quintanilla, David L. Hoffman, Karen L. Bentley, Jeffery D. Molkentin, Shey-Shing Sheu, George A. Porter.

Little is known about cardiac energetics and mitochondrial function in the embryo, and we hypothesize that the mitochondrial permeability transition pore (mPTP) controls mitochondrial structure and function during embryonic cardiac development and is critical for normal myocyte differentiation and cardiac morphogenesis. To test this hypothesis, we examined mitochondrial structure and function in cultured myocytes and whole heart using light and electron microscopy. Mitochondria of embryonic day (E) 9.5 ventricular myocytes displayed less dense cristae and were shorter in length and less branched. By E13.5, mitochondria had abundant cristae, were longer, branched and

networked, and were more closely associated with the contractile apparatus. Functional measurements demonstrated dramatic increases in mitochondrial membrane potential, an increased reliance on complex I, and a decrease in oxidative stress as the heart developed. These structural and functional data suggested an increase in inner mitochondrial membrane permeability, and closure of the mPTP using cyclosporin A or cyclophilin-D null embryos caused premature maturation of mitochondrial structure, mitochondrial function, and myocyte differentiation. Furthermore, long term opening of the mPTP using carboxyatractyloside after E9.5 inhibited mitochondrial maturation and myocyte differentiation. Taken together, these data suggest a critical role of the embryonic mPTP as a mediator of mitochondrial maturation and cardiac differentiation.

241-Pos Board B41

Mitochondrial Motility During Vascular Smooth Muscle Proliferation

Susan Chalmers, Paul Coats, Christopher D. Saunter, John G. McCarron.

Mitochondrial function is critical for multiple facets of cellular function, including ATP production and Ca^{2+} handling. Subcellular location of the organelle is important for function. For example, in smooth muscle, mitochondria modulate Ca^{2+} signals arising from the inositol-trisphosphate-sensitive channels (IP_3R), even at the level of Ca^{2+} puffs, revealing a close physical relationship between IP_3R and mitochondria. This relationship seems incompatible with rapid free movement of mitochondria-observed in several cell types. Here we report mitochondria in freshly-isolated smooth muscle cells lacked directed motion and Brownian-like movement was limited so that no displacement of individual organelles occurred. The movement did not change by disruption of actin polymerisation (latrunculin-B, 10 μM), inhibition of microtubule polymerisation (nocodazole, 10 μM) or removal of Ca^{2+} from the extracellular solution. In intact pressurised (40 mmHg) cerebral arteries, individual mitochondria within the smooth muscle were largely stationary; however in a small but significant number of cells mitochondria displayed directed movements. When these arteries were maintained in conditions which promote cell proliferation, an increased number of cells showing moving mitochondria and an increase in the extent of movement occurred. We postulated that those smooth muscle cells displaying motile mitochondria may be proliferative, as downregulation of the mitochondrial tether mitofusin-2 has been reported in proliferative vascular disease. In support, within days of allowing freshly-isolated cerebral artery smooth muscle cells to proliferate in culture, extensive mitochondrial motility developed. A spectrum of movements was observed: long- and short-distance directed movements, wiggling, looping, extension, retraction, and Brownian-like diffusion. Therefore, when isolated smooth muscle cells are allowed to proliferate, mitochondria switch from being static, with no observed motion (that suggests physical confinement), to being highly mobile and unconfined organelles. This increased mitochondrial mobility may contribute to the altered Ca^{2+} signalling observed in proliferative smooth muscle.

Protein Structure

242-Pos Board B42

Assembly of Reverse Transcriptase from Feline Immunodeficiency Virus

Labe Black, J.B.A. Ross, Cate Burgess, Mary Poss, Martin Lawrence, Stephanie Omburo.

The reverse transcriptase (RT) of feline immunodeficiency virus (FIV), like the enzyme of the lentivirus that infects humans (HIV), is a heterodimer composed of 51 kDa and 66 kDa subunits. FIV RT exhibits different error and recombination rates in different host species_RT in cougars shows significantly lower error and recombination rates than RT in house cats. Barkley and coworkers (Ignatov et al., (2005) Biochemistry 44, 5346-5356), using analytical ultracentrifugation (AUC), have shown that although HIV RT tends to assemble as the heterodimer, there is also significant assembly as homodimers. To determine how the FIV RT assembly properties might correlate with error and recombination rates, we are studying the subunit assembly of RT from cougars and house cats by AUC, fluorescence correlation spectroscopy, and pressure perturbation.

243-Pos Board B43

Flaviviral Helicases: Structure, Function, Inhibition and Dynamics

Eloise Mastrangelo, Martino Bolognesi, **Mario Milani**.

Flaviviruses are ssRNA viruses causing several human diseases such as Dengue and Yellow fever. Helicases (Hel) are motor proteins involved in nucleotide remodeling. During viral replication the new formed dsRNA (template+daughter) is unwound by Hel. A molecular picture describing how the chemical energy derived from ATP hydrolysis is transformed into mechanical actions required for dsRNA unwinding is still missing.

Flaviviral Hel is a monomeric protein composed of three domains hosting an internal cleft that bind ssRNA. From the crystal structure of Kunjin virus helicase (K-Hel; Mastrangelo et al., 2007) we hypothesized that the ssRNA entrance site was located between domains II and III. We evaluated such site as an attractive target for protein inhibition. Using in silico docking we identify a new compound, proved to be active against flaviviruses in cell culture (patent EP09174368).

Normal mode analysis of K-Hel suggested a 'scissor-like' domain rearrangement likely involved in protein activity. Accordingly, the structures of Kokobera Hel in two crystal forms showed open and closed conformations of the ssRNA access site (unpublished results).

In order to investigate such dynamical features we performed two molecular dynamics simulations of Dengue Hel in the presence and absence of ATP. The dynamics showed that ATP induces the closure of the ssRNA access site. The mechanical feature of the signal transmission between ATP binding site and ssRNA access site, located about 30 Å apart, are described and a novel role of Hel motif V is underlined. Preliminary SAXS experiment confirmed the role of ATP in inducing protein closure, showing a slightly reduced radius of gyration. Protein mutants will be produced to analyze the 'transmission shaft' from ATP binding site to ssRNA access site.

244-Pos Board B44

Simulation and Prediction of the Active Conformation During Autophosphorylation in Two-Component Signaling

Alexander Schug.

Bacteria use two-component signal transduction systems (TCS) to sense and react to external stimuli. A membrane bound sensor histidine kinase (SK) detects an environmental stimulus and forms a complex with a transcription factor/response regulator (RR) transferring a phosphoryl group to mediate a cellular response. The complex is ruled by transient interactions, and only recently was a complex structure of a HK/RR pair structurally resolved [1]. Concurently, we predicted this complex structure in high agreement (3.5 Å RMSD) with the experimental work by a mixed theory approach of molecular dynamics and statistical genomic analysis [2,3]. Based on this theoretical work, it is now possible to predict the structural changes occuring during autophosphorylation. Direct coupling analysis [3] identifies innerprotein contacts formed between the HisKa and ATP-bindind domain which are not realized in the crystal structure. This information can be used for molecular dynamics simulations to identify a putable active conformation adopted during autophosphorylation.

[1] Casino P, Rubio V, and Marina MA. *Structural Insight into Partner Specificity and Phosphoryl Transfer in Two-Component Signal Transduction*. Cell 139 (2009), 325-336

[2] Schug A, Weigt M, Onuchic JN, Hwa T, and Szurmant H. *High-resolution protein complexes from integrating genomic information with molecular simulation*. Proc Nat Acad Sci USA (2009) 106, 22124-22129

[3] Weigt M, White RA, Szurmant H, Hoch JA, and Hwa T. *Identification of direct residue contacts in protein-protein interaction by message passing*. Proc Nat Acad Sci USA (2009) 106, 67-72

245-Pos Board B45

Insights into Halophilic Protein Stability via the Generalized Born Model

Charles J. Baker, Alexey Onufriev, Biswarup Mukhopadhyay.

In contrast to proteins from bacteria that live in low salinity environments, proteins from halophiles, bacteria that live in inhospitable environments such as the Dead Sea, have been shown to be stabilized at high salt concentration and actually denature at 'normal' salinity. Furthermore, it is known that halophilic proteins have a higher number of negatively charged amino acids than non-halophilic proteins, particularly at their surfaces. Neither the reason for the halophilic proteins' net surface charge nor its stabilization at high salinity is completely understood. In this study, randomly selected halophilic and non-halophilic proteins are investigated within the context of the Generalized Born model of implicit solvation. This analysis provides insight into how the charged nature of these halophilic proteins can increase their stability at high salinity while causing them denature at low salinity. Further insight is then gained via a detailed study of halophilic and non-halophilic protein homologues.